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Formulation and Antimicrobial Activity of Nanoparticles of Leaf Extract of *Albizia amara*

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Abstract

Silver nanoparticles (AA-AgNPs) were made from *Albizia amara* leaf extract using green synthesis. The nanoparticles were characterized by X-ray diffraction spectroscopy to identify the crystalline/amorphous nature). According to the XRD pattern, the (111), (200), (220), and (222) planes have Bragg's diffraction peaks at 38.05°, 44.17°, 64.44°, and 77.32°, respectively. The face-centered cubic lattice structure of nanoparticles is thus connected to these angles. The antimicrobial activity of *Albizia amara* AgNPs by Disc diffusion assay. *Staphylococcus aureus*, or *E. Coli*, was discovered to be between 50 and 350 g/ml. Escherichia coli, one of these strains, was shown to be the most susceptible with a MIC value of 120 g/ml whereas Staphylococcus aureus, another strain, was discovered to be very resistant to AA AgNPs with a MIC value of 140 g/ml.

Keywords: Silver nanoparticle, green synthesis, *Albizia amara*, antioxidant activity

Introduction

Throughout history, several societies have utilized medicinal herbs for their healing qualities [1-2]. Medicinal plants have been used historically from prehistoric times, and knowledge of these plants has been passed down through the centuries [3-4].

Medicinal plants and other natural ingredients are used in traditional herbal remedies. Plant components such as leaves, flowers, stems, roots, and seeds are used to make herbal remedies. These plant materials have active ingredients with medicinal qualities [6-8].

Antibiotic resistance, a global health crisis, emerges as bacteria adapt and evolve to survive the drugs designed to eliminate them. Overuse and misuse of antibiotics accelerate this process, rendering once-effective treatments ineffective. This phenomenon jeopardizes our ability to combat infectious diseases, leading to prolonged illnesses, increased mortality rates, and elevated healthcare costs. Urgent action, including prudent antibiotic use, robust surveillance, and the development of novel antimicrobial agents, is imperative to mitigate the dire consequences of antibiotic resistance and safeguard public health for generations to come [9-10].

Albizia amara is a tree in the family Fabaceae. Its range includes southern and Eastern Africa, from South Africa to Sudan and Ethiopia. It is also found in India and Sri Lanka. The leaves and flowers are used for treatment of boils and ulcers. The leaf is also used for treatment of erysipelas. Paste of leaf and rootbark is used to cure both skin diseases and poisonous bites. The seeds are regarded as astringent and used in the treatment of piles, diarrhea and gonorrhea. The flowers are used as a remedy for cough, ulcers, dandruff and malaria [11-13].

This research aims to uncover the mechanisms of action, assess the efficacy, and explore the antimicrobial activity of silver nanoparticles of leaf extract of *Albizia amara.*

Material and Methods

Collection and Authentication of *Albizia amara leaves* of plant

Albizia amara (Fabaceae) leaves are collected from the Medicinal Garden of ICMR- National Institute of Traditional Medicine. RMRC ICMR layout, Nehru Nagar Belagavi, Karnataka and authenticated (Accession number: RMRC-1739) by Dr. Harsha Hegde, Taxonomist ICMR Belagavi, Karnataka, India

Preparation of aqueous leaf extract

We carefully weighed about 20 grams of recently harvested *Albizia amara* leaves. The leaves were then thoroughly washed twice under running tap water to remove any remaining dust and muck. After that, Milli Q water was used to rinse the leaves. After being roughly cut into tiny pieces, the leaves were boiled for 30 minutes at 60 °C in 100 milliliters of Milli Q water. The mixture was brought to a boil to form an extract, which was then cooled to room temperature and filtered twice: once through regular filter paper and once through Whatman No. 1 filter paper. Four degrees Celsius was the ideal temperature for the filtered extract to be kept [14-16].

Synthesis of silver nanoparticles

Silver nanoparticles will develop as a result of the reduction of Ag+ ions to Ag0 in 100 ml of 1 mM silver nitrate solution at 70 °C and constant stirring with a magnetic stirrer. This was achieved by adding 10 ml of aqueous AA-extract. After adding 1% w/v NaOH solution to bring the reaction mixture's pH to 8, it was then allowed to incubate for 24 hours in the dark at Centrifugation was used to separate the resulting AA-AgNPs for 30 minutes at 10,000 rpm (Kubota Corporation, Japan). After that, pellets were centrifuged three more times to get rid of any remaining extract after being re-suspended in Milli Q water. The effects of temperature, pH, silver nitrate concentration, extract to silver nitrate ratio, and reaction incubation time were then optimized [17-21].

Characterization of *Albizia amara* AgNPs

Phytochemical analysis of extract was done and reported in our previously published paper. Ultraviolet-visible spectral analysis, Principle of Particle size and Zeta Potential analysis, Transmission electron microscopy (TEM) study has been reported in our previous paper [22].

X-Ray Diffraction (XRD)

The X-ray diffraction (XRD) pattern of AA-AgNPs synthesized through green methods was captured using a Rigaku model Smart Lab 3kW X-ray diffractometer from Japan. The instrument was equipped with a CuKα radiation source and a nickel monochromator filter. The XRD scan was conducted within a 2θ range of 20° to 80°. To record the pattern, a dispersion solution of the nanoparticles was placed onto a microscope glass slide and subsequently dried at 60 °C using a hot air oven. This drying process resulted in the formation of a thin layer of the nanoparticle solution on the slide. The scanning of the XRD pattern was performed at a steady rate of 2° per minute.

Antimicrobial Activity

The antimicrobial activity of *Albizia amara* AgNPs by Disc diffusion assay as described in the literature. The pure cultures of bacterial and fungal pathogens were subculture in Brain heart infusion broth (BHI) respectively. Wells of 5mm diameter were made on Brain heart infusion agar (BHI) plate. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, 50µl of the sample of *Albizia amara* AgNPs, *Albizia amara* extract, silver nitrate, standard antibiotic (Ciprofloxacin for antibacterial and Fluconazole for fungal activity) was pipetted onto each well on all plates. The plates were incubated at 37ºC for 24 hr. After incubation the different levels of zone of inhibition of bacteria and fungi were measured [19-21].

Determination of minimum inhibitory concentration (MIC)

In a 96-well microtitre plate using the two-fold dilution technique, the MIC and MBC of *Albizia amara* AgNPs, *Albizia amara* extract, silver nitrate, and standard antibiotic were determined against test bacteria and fungi. (100µg/ml-0.20µg/ml). It was cultivated in its purest form. These organisms were produced as a suspension in brain heart infusion broth (BHI). To carry out the MIC activity, this prepared suspension of the appropriate bacteria and fungus was utilised. The lowest concentration of an antibacterial growth was noted as the (MIC) [19,21].

Determination of minimum bactericidal concentration (MBC)

The two-fold dilution of varying concentration was selected for the treatment of MBC. The overnight grown culture from MIC was taken streaked on BHI agar plates from each treated concentration to determine the MBC [19,21].

Results and Discussion

The crystalline/amorphous nature of synthesised silver nanoparticles was identified by X-ray diffraction spectroscopy (XRD). Figure 1 depicts the AA-AgNPs' XRD pattern. According to the XRD pattern, the (111), (200), (220), and (222) planes have Bragg's diffraction peaks at 38.05°, 44.17°, 64.44°, and 77.32°, respectively. The face-centered cubic lattice structure of nanoparticles is thus connected to these angles. Data from an XRD study were used to further validate the SAED findings.

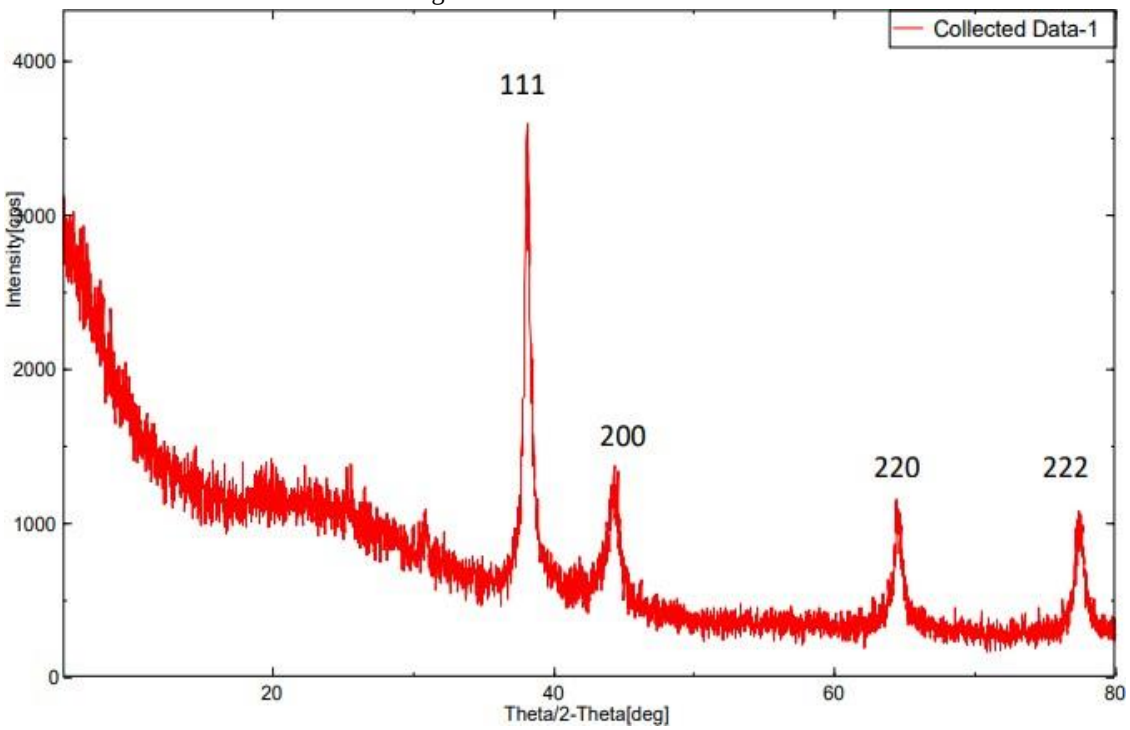

Figure 1: X-ray diffraction spectrum of AA-AgNPs

***In-vitro* Assessment of Antibacterial Activity of AA-AGNPs**

In Table 1, the MIC values of the AAAgNPs against the strains of test pathogens are listed. A gramme +ve bacterial strain of Staphylococcus aureus (ATCC 25923), a one gramme -ve bacterial strain of *Escherichia coli*, and other microorganisms were discovered to be resistant to the AA-AgNPs. The range of the MIC for AA AgNPs against pathogens was discovered to be 50–350 g/ml. Escherichia coli, one of these strains, was shown to be the most susceptible with a MIC value of 80 g/ml whereas *Staphylococcus aureus*, another strain, was discovered to be extremely resistant to AA AgNPs with a MIC value of 100 g/ml. The minimum bactericidal concentration (MBC) is the lowest concentrationof an antibacterial agent needed to kill a bacterium over a given amount of time, undera

particular set of circumstances, and did not exhibit any apparent signs of microorganism growth when streaked on the agar plates. The MBC values for Escherichia coli. Staphylococcus aureus, or Coli, was discovered to be between 50and 350 g/ml. Escherichia coli, one of these strains, was shown to be the most susceptible with a MIC value of 120 g/ml whereas Staphylococcus aureus, another strain, was discovered to be very resistant to AA AgNPs with a MIC value of 140 g/ml. These numbers line up with the MIC and MBC in Table 1. The greatest concentration to kill the various pathogens was determined to be 140 g/ml by taking into account the findings of MIC and MBC, and this concentration was selected for future research of zone of inhibition. The formation of a clear zone around the AgNPs disc suggests that the AA AgNPs possessed good antibacterial activity and was able to inhibit the growth of pathogens when compared to AA extract, *Albizia amara* silver nanoparticles, Rifampicin, and Ampicillin. These findings were obtained using the agar diffusion method. The zone of inhibition evaluated for various bacterial strains of AA AgNPs is as follows: 11.33mm for *Staphylococcus aureus*, and 13.33 mm for *Escherichia coli*.

AA AgNPs demonstrated the considerable antibacterial activity of the bacterium when compared to the AA extract, Rifampicin, and Ampicillin by comparing the distinct zones of inhibition. The possibility that AgNPs adhere to the surface of the cell membrane and disrupt the permeability and respiration processes of the cell is one explanation for theantibacterial action of silver. AgNPs' smallest particle sizes, which range from 10 to 24 nm, may allow them to enter the bacterium in addition to interacting with the membrane's surface.

Table 1: Antibacterial properties of samples in MIC and MBC

Sl.	Sample name	*Staphylococcus aureus*		*Escherichia coli*	
		MIC (µg/ml)	**MBC(µg/ml)**	**MIC(µg/ml)**	**MBC(µg/ml)**
1	Albiziaamara Extract	250	300	250	300
2	*Albizia amara* silver nanoparticles	100	140	80	120
3	Std. kanamycin	30	60	30	70

Conclusion

Metallic nanoparticles have become a popular drug delivery technique over traditional therapy. This work synthesizes silver nanoparticles from *Albizia amara* leaf aqueous extract and screened for antimicrobial study. Among this strains *Escherichia coli* found to be most sensitive with an MIC value of 120 µg/ml while, *Staphylococcus aureus* was found to be highly resistant against AA AgNPs with a MIC value of 140µg/ml. These values are accordance with MIC and MBC. Byconsidering the results of MIC and MBC it is found that 140 µg/ml was found to be the maximum concentration to kill the different pathogens and their concentration was chosenfor further studies of zone of inhibition. To boost the antimicrobial activity and encourage more metallic nano particles can be added to the preparation of *Albizia amara*.

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